**Material and Methods**

**Material:**

**A- Animals**

This study was carried out on sixty-six adult albino rats (forty-four females and twenty-two male albino rats) weighing 200- 250 gm each. The animals were obtained from the animal house of the faculty of veterinary medicine, Benha University. The experimental rats were kept in metal cages with proper ventilation and humidity and were being under complete healthy conditions in the form of clean environment, good ventilation, controlled room temperature (25±2̊c), on 12 hours light/dark cycle with free access to water and balanced diet. All aspects of the research have complied with the protocols approved by the local ethical committee of the Faculty of Medicine, Benha University.

**Mating the animals and timing of pregnancy**

After acclimatization to the laboratory conditions for one week, each 2 female rats were kept with one male rat and left overnight in a separate cage to allow mating. Early in the next morning, copulation was confirmed by vaginal smear. Copulation was confirmed in thirty-six female albino rats. The gestational day zero was defined when spermatozoa were observed in a smear of the vaginal contents. Subsequent days of gestation were numbered accordingly **(Adu and Yeboah, 2000)**.

**B-Drugs**

**1-The Pregabalin (Lyrica)**: It was in the form of capsules of 75 mg concentrations. It was obtained from the Pfizer company. It was used in an effective dose of 300 mg/kg body weight twice a day. The dose for the adult rat weight 250 gm was 75 mg dissolved in 1ml distilled water. This dose was given orally twice a day by a gastric tube **(Salih et al., 2014)**.

**2-** **L-carnitine (carnitol)**: It was obtained from Global Pharmaceutical Industries. It was used in capsule form and each capsule contains 500 mg carnitine. It was given orally by a gastric tube once daily to the treated groups at a dose of 500 mg/kg body weight of the rat. So the adult rat weight 250 gm, needs 125mg/day. The dose was given by dissolving one capsule in (2ml) distilled water so each 1 ml contains 250 mg carnitine **(Czeczot and Scibior, 2005)**.

**Methods:**

Thirty-six pregnant rats used in this study and were divided into two main groups:

**Group A**- **Consist of eighteen, pregnant rats for the prenatal study**.

It was subdivided into:

**A (1):** **Control group of 6 pregnant rats:**

Each pregnant rat had received a dose of 1ml of distilled water twice/day by a gastric tube daily during pregnancy period. Three of them were sacrificed at the 18th day of gestation and the other three pregnant rats were sacrificed at the 20th day of gestation.

**A (2):** **Treated group of twelve pregnant rats:**

This group was subdivided into two subgroups as follow:

**A (2a)** - **Pregabalin treated group of six pregnant rats;**

Each pregnant rat was treated daily with the Pregabalin drug at a dose of 300 mg/kg body weight, twice/day orally by a gastric tube during the pregnancy period. Three of these pregnant rats were sacrificed at the 18th day of gestation while the other three pregnant rats were sacrificed at the 20th day of gestation.

**A (2b)** - **Pregabalin and L-carnitine treated group of six pregnant** **rats;**

Each pregnant rat was treated daily with the Pregabalin drug at a dose of 300 mg/kg body weight, twice/day orally by a gastric tube and L-carnitine drug in a single dose of 500 mg/kg body weight orally by a gastric tube during the pregnancy period. Three of these pregnant rats were sacrificed at the 18th day of gestation and the other three pregnant rats were sacrificed at the 20th day of gestation.

**Group B**- **Consist of eighteen, pregnant rats for postnatal study.**

It was subdivided into:

**B (1)** - **Control group of six pregnant rats.**

Each pregnant rat had received an oral daily dose of (1ml) of distilled water twice/day during the pregnancy period and was remained until gave birth. The mothers had continued to receive the dose of distilled water during the lactating period. The pups were kept on breastfeeding with their mothers in healthy conditions from the first day of life until the end of the weaning period at day fourteen. Some pups were sacrificed after 24 hours after birth while others were sacrificed at the 7th and the 14th days after birth. After weaning, the other pups had received the dose of distilled water according to their body weight and were sacrificed at the 28th and the 60th days after birth.

**B (2)** - **Treated group of twelve pregnant rats**

This group was subdivided into two subgroups as follow:

**B (2a)** - **Pregabalin treated group of 6 pregnant rats;**

Each pregnant rat was treated daily with the Pregabalin drug at a dose of 300 mg/kg body weight, twice/day orally by a gastric tube throughout the pregnancy period and remained until gave birth. The mothers continued to receive the same dose of the drug during the lactating period. The pups were kept on breastfeeding with their mothers in healthy conditions from the first day of life until the end of the weaning period at day fourteen. Some pups were sacrificed after 24 hours after birth. Other pups were sacrificed at the 7th day and the 14th day. After weaning, the other pups had received the dose of the Pregabalin drug (300 mg/kg body weight, twice/day) daily and were sacrificed at the 28th day and the 60th day after birth.

**B (2b)**- **Pregabalin and L-carnitine treated group of six pregnant rats;**

Each pregnant rat was treated with the Pregabalin drug at a dose of 300 mg/kg body weight, twice/day orally by a gastric tube throughout the pregnancy period and remained until gave birth. The same pregnant rats were treated with L-carnitine drug in a single dose of 500 mg/kg body weight orally by a gastric tube. The mothers continued to receive the same dose of drugs during the lactating period. The pups were kept on breastfeeding with their mothers in healthy conditions from the first day of life until the end of the weaning period at day fourteen. Some pups were sacrificed after 24 hours after birth. Other pups were sacrificed at the 7th day and the 14th day after birth. After weaning, the other pups received the dose of the Pregabalin drug (300 mg/kg body weight, twice/day) and the dose of L-carnitine drug (500 mg/kg body weight, once/day) orally by a gastric tube and were sacrificed at the 28th day and the 60th day.

**Collection of the prenatal specimens:**

The pregnant rats of the prenatal groups were anesthetized using ether inhalation at the 18th and 20th gestational days. An abdominal midline incision was performed and the two uterine horns were exposed. The fetuses were extracted from the placental sacs. A part of the skull of the fetuses was removed for the extraction of the whole brain and the cerebellum was isolated from the brain carefully by an incision along the dorsal aspect, under a dissecting microscope. The specimens were prepared for histological studies.

After the end of the experiment, the rats were eliminated by incineration in Benha University incinerator.

**Collection of the postnatal specimens:**

The rats of postnatal groups aged 1 day, 7 days, 14days, 28 days and 60 days after birth were given deep anesthesia by ether inhalation. A midline incision was made, starting from the dorsal aspect of the upper part of the neck and was extended to the head. After reflecting the scalp, the dorsal calvarium was removed in small chips, taking care not to damage the underlying brain tissue. The brain was carefully dissected out. The cerebellum was slightly lifted up and the cerebellar peduncles were cut, thus separating the cerebellum from the brain stem. The two cerebellar hemispheres were separated by a sagittal cut passing through the vermis. The tissues were washed with normal saline and fixed in the fixative. Tissue processing was done for histological studies.

After the end of the experiment, the rats were eliminated by incineration in Benha University incinerator.

**Histological techniques**:

***For Light Microscopic Study;***

The specimens were fixed at 10% neutral- buffered formalin, dehydrated through alcohols, cleared in xylene. The tissue was impregnated with paraffin wax and then embedded in paraffin wax for preparation of paraffin blocks. Sagittal histological sections of 4-5 μm thickness were taken, de-waxed, hydrated, mounted on glass slides and stained with Haematoxylin and Eosin stain and special stain (Silver stain) and then covered with coverslips **(Bancroft and Gamble, 2008)**. The slides were viewed and photographed by the light microscope equipped with an automatic photomicrographic camera systemin the Anatomy and Pathology departments, Faculty of Medicine, Benha University.

***For Electron Microscopic Study;***

The extracted specimens were cut into slices 1 μm thick (small pieces). They were fixed in 2.5% glutaraldehyde for 24 hours. Specimens were Washed 3 times (5 mins each) with phosphate buffer at 4°C, then post-fixed in 1% osmium tetroxide at room temperature for 30 minutes. Specimens were dehydrated in ascending grades of ethyl alcohol, 50% alcohol for 30 min, 70% alcohol for 15 min each, 80% alcohol for 15 min, 90% alcohol for 15 min, in 100% alcohol for 30 min each, then embedded in a capsule by using an embedding mixture. The capsules, then polymerized in a temperature controlled oven at 60̊ C for 48 hours. The polymerized block was trimmed into a pyramid. After that, Semithin sections (1μm) thickness were cut using a glass knife and were stained with 1% toluidine blue stain dissolved in 1% borax for 30- 60 seconds at 60̊ C, then examined with a light microscope to choose the selected areas. Ultrathin sections (50 nm) thick were obtained from the selected blocks using a diamond knife and mounted on copper grids. The grids were stained with uranyl acetate for 20 minutes, then by lead citrate for 10 minutes, then left to dry and stored in a grid box till examined by an electron microscope. **(Bozzola and Russell, 1999)**.This processing was done in Tanta Faculty of Medicine. After that, the grids were examined by Philips 201-transmission electron microscope at 60-80 KV in the transmission electron microscope unit at Tanta Faculty of Medicine, Tanta University. Finally, the electron micrographs were taken from the selected areas.